

### Claims

1. A method of forming a structure, preferably a hole or cavity or channel, in a region of an electrically insulating substrate, comprising the steps:

- a) providing an electrically insulating substrate,
- b) applying, by means of a voltage supply, a voltage across a region of said electrically insulating substrate, said voltage being sufficient to give rise to a significant increase in electrical current through said region and to a dielectric breakdown (DEB) through said region,
- c) applying energy, preferably heat, to said substrate or said region only so as to increase the temperature of said region, said energy, preferably heat, originating either from an energy or heat source or from components of said voltage applied in step b), said energy, preferably heat, being applied so as to reduce the amplitude of voltage required in step b) to give rise to said current increase and/or to soften the material of said region,

wherein step b) is performed and, preferably, ended using an electronic feedback mechanism operating according to user-predefined parameters, said electronic feedback mechanism controlling the properties of said applied voltage and/or of said electrical current.

2. The method according to claim 1, wherein said electronic feedback mechanism causes an end of step b) within a user-predefined period after onset of said dielectric breakdown, said onset preferably being an increase in the number of charge carriers per unit time, by a factor of 2, preferably by at least one order of magnitude.

3. The method according to any of the foregoing claims, wherein said significant increase in electrical current is an increase in the number of charge carriers per unit time, by a factor of 2, preferably by at least one order of magnitude.

4. The method according to any of claims 2 – 3, wherein said electronic feedback mechanism causes said end of step b) to occur – with or without a preset delay – at the time when said

electrical current has reached a threshold value, preferably in the range of 0.01 to 10 mA, or at the time, when an increase in electrical current,  $(dI/dt)$ , has reached a threshold value, preferably equal or larger than 0.01 A/s.

5. The method according to any of the foregoing claims, wherein said electronic feedback mechanism is fast enough to be able to cause an end of step b) within a period in the range of from 1 ns to 100 ms, preferably from 1 ns to 100 us, more preferably 100 ns to 10 us, after onset of said dielectric breakdown, or within the aforementioned period after said increase in electrical current has reached said threshold value.

6. The method according to claim 5, wherein said electronic feedback mechanism causes an end of step b) within a period in the range of from 100 ns to 10 s, preferably 100 ns to 1 sec, after onset of said dielectric breakdown or after said increase in electrical current has reached said threshold value.

7. The method according to any of claims 2 – 6, wherein said end of step b) occurs without any intervention by a user once step b) has been initiated.

8. The method according to any of the foregoing claims, wherein said electronic feedback mechanism comprises a current and/or voltage analysis circuit such as a trigger circuit, alone or as part of a user-programmed device, such as a computer, said current and/or voltage analysis circuit being capable of controlling voltage supply output parameters, and/or being capable of controlling said energy or heat source, if present.

9. The method according to any of the foregoing claims, wherein steps b) and c) occur concomitantly.

10. The method according to any of the foregoing claims, wherein step c) is performed under control of a user, preferably by use of said electronic feedback mechanism.

11. The method according to claim 10, wherein said control of a user involves definition or regulation of the amount and/or the duration of said energy, preferably heat, applied to said region in step c).

12. The method according to any of the foregoing claims, wherein said electronic feedback mechanism provides for a regulation of amplitude and/or duration of said voltage and/or said current.
13. The method according to any of the foregoing claims wherein said voltage is in the range of  $10^2$  V to  $10^6$  V, preferably in the range of from  $10^3$  V –  $10^5$  V.
14. The method according any of the foregoing claims, wherein step c) is initiated before step b).
15. The method according to any of the foregoing claims, wherein step c) is continued after step b) has been ended.
16. The method according to any of the foregoing claims, wherein step b) occurs by the placement of electrodes at or near said region, preferably by placing one electrode on one side of that substrate and by placing another electrode on another side of said substrate, and by application of said voltage across said electrodes.
17. The method according to any of the foregoing claims, wherein, at the beginning of step b), said voltage is increased in amplitude up to a value, at which an increase in electrical current through said region occurs and/or where a dielectric breakdown (DEB) through said substrate occurs and/or where an electric arc occurs.
18. The method according to any of the foregoing claims, wherein said current flows along a current path through said substrate region and changes viscosity and/or stiffness and/or brittleness of said substrate along and near said current path.
19. The method according to claim 18, wherein said current softens and/or melts and/or evaporates said substrate along and near said current path, and/or wherein said current and/or said applied voltage cause the removal of substrate material along and near said current path, preferably by evaporation, ejection, electrostatic attraction or a combination thereof.

20. The method according to any of claims 18 – 19, wherein step b) does not lead to a breakage of said substrate, and wherein, preferably, said current, current increase and voltage parameters are limited by a user to values, said values being more preferably determined experimentally for each substrate material and/or substrate material class, at which values no breakage of said substrate is caused.
21. The method according to any of the foregoing claims, wherein said applied voltage is purely DC.
22. The method according to any of claims 1 - 20, wherein said applied voltage is purely AC.
23. The method according to any of claims 1 - 20, wherein said applied voltage is a superposition of AC and DC voltages.
24. The method according to any of claims 22 - 23, wherein the frequency of said applied AC voltage is in the range of from  $10^2$  to  $10^{12}$  Hz, preferably in the range of from  $5 \times 10^2$  to  $10^8$  Hz, more preferably  $1 \times 10^3$  to  $1 \times 10^7$  Hz..
25. The method according to any of claims 22 – 24, wherein said AC voltage is applied intermittently, preferably in pulse trains of a duration in the range of from 1 ms to 1000 ms, preferably 10 ms to 500 ms, with a pause in between of a duration of at least 1 ms, preferably of at least 10 ms.
26. The method according to any of claims 22 - 25, wherein said applied AC voltage is used for performing step c).
27. The method according to any of claims 22 - 26, wherein said applied AC voltage has parameters (e.g. amplitude, frequency, duty cycle) which are sufficient to establish an electric arc between a surface of said substrate and said electrodes.
28. The method according to claim 27, wherein said electric arc is used for performing step c).

29 The method according to any of claims 26 - 28, wherein said applied AC voltage leads to dielectric losses in said region of said substrate, said dielectric losses being sufficient to increase the temperature of said region.

30. The method according to any of claims 22 – 29, wherein the frequency of said applied AC voltage is increased to reduce deviations of the current path from a direct straight line between the electrodes.

31. The method according to any of claims 22 – 30, wherein the frequency of said applied AC voltage is increased to minimize the possible distance between neighbouring structures, preferably neighbouring holes.

32. The method according to any of claims, wherein, in step c), energy, preferably heat, is applied to said region so as to decrease the voltage amplitude required to initiate dielectric breakdown across this region.

33. The method according to any of the foregoing claims, wherein in step c), heat is applied to said region of said substrate using a heated electrode or a heating element placed near by the electrode.

34. The method according to claim 33, wherein said heated electrode is an electric heating filament and is also used to apply said voltage to said region in step b).

35. The method according to any of the foregoing claims, wherein, in step c), heat is applied to said region of said substrate additionally or only by using an external heat source, such as a laser or other focussed light source, or by using a gas flame.

36 The method according to any of the foregoing claims, wherein, in step c), heat is applied to said region of said substrate by applying an AC voltage to said region.

37. The method according to claim 36, wherein said AC voltage is applied to said region by electrodes placed on opposite sides of said substrate, preferably at least one electrode being placed on one side of said substrate and at least one electrode being placed on another side of said substrate.

38. The method according to claims 37, wherein said electrodes placed on opposite sides of said substrate are also used for performing step b).

39. The method according to any of claims 36 - 38, wherein said AC voltage is sufficient to cause dielectric losses in said region of said substrate leading to an increase in temperature in said region.

40. The method according to claim 39, wherein said AC voltage is in the range of  $10^3$  V -  $10^6$  V, preferably  $2 \times 10^3$  V -  $10^5$  V, and has a frequency in the range of from  $10^2$  Hz to  $10^{12}$  Hz, preferably in the range of from  $5 \times 10^2$  to  $10^8$  Hz, more preferably  $1 \times 10^3$  to  $1 \times 10^7$  Hz.

41. The method according to any of the foregoing claims, wherein said structure being formed is a hole having a diameter in the range of from 0.01  $\mu\text{m}$  to 50  $\mu\text{m}$ , preferably 0.1  $\mu\text{m}$  to 10  $\mu\text{m}$ , and more preferably 0.3  $\mu\text{m}$  to 5  $\mu\text{m}$ .

42. The method according to any of claims 1 - 40, wherein said structure being formed is a cavity having a diameter in the range of from 0.1  $\mu\text{m}$  to 100  $\mu\text{m}$ .

43. The method according to any of claims 1 - 40, wherein said voltage is applied by electrodes placed on opposite sides of said substrate, and said structure being formed is a channel-like structure obtained by a relative movement of said electrodes in relation to said substrate.

44. The method according to any of the foregoing claims, wherein said structure, preferably said hole has an aspect ratio greater than 1, preferably greater than 5.

45. The method according to any of the foregoing claims, wherein said electrically insulating substrate is selected from a group comprising carbon-based polymers, such as polypropylene,

fluoropolymers, such as Teflon, silicon-based substrates, such as glass, quartz, silicon nitride, silicon oxide, silicon based polymers such as Sylgard, semiconducting materials such as elemental silicon.

46. The method according to any of the foregoing claims, wherein said region where a structure is to be formed, has a thickness in the range of from  $10^{-9}$  m to  $10^{-2}$  m, preferably  $10^{-7}$  m to  $10^{-3}$  m, more preferably  $10^{-5}$  m to  $5 \times 10^{-4}$  m, most preferably  $>10^{-6}$  m.

47. The method according to any of the foregoing claims, wherein said substrate is provided in step a) within a material (solid, liquid or gas) that reacts with a surface of said substrate during steps b) and/or c).

48. The method according to any of the foregoing claims, wherein, after formation of said structure, a surface of said structure is smoothed by further application of heat, preferably by application of heat through step c).

49. The method according to any of the foregoing claims, wherein, after formation of said structure, its shape is subsequently altered by further application of heat, preferably by application of heat through step c).

50. The method according to any of claims 48 – 49, wherein said further application of heat occurs by an electric arc formed between two electrodes, preferably two electrodes which are used for performing step b).

51. The method according to any of the foregoing claims, wherein said electrically insulating substrate is a substrate, wherein dielectric breakdown occurs using a small voltage, in the absence of additional heat or energy, preferably using a voltage in the range below 10 kV, and wherein step c) is omitted altogether.

52. A device for forming a structure in a region of an electrically insulating substrate, preferably for performing the method according to any of the foregoing claims, comprising at least two electrodes connected to a voltage supply, which can be controlled by a trans-

substrate current, and means to apply energy, preferably heat, to said substrate, wherein said means is one electrode or said at least two electrodes or is an additional heat source.

53 The device according to claim 52, wherein there is no additional heat source.

54. The device according to any of claims 52 - 53, further comprising means to receive and hold said electrically insulating substrate while said structure is being formed in said region of said substrate.

55. The device according to any of claims 52 - 54, further comprising an analysis and control unit, which may be part of said voltage supply, comprising a current and/or voltage analysis circuit such as a trigger circuit, alone or as part of a user-programmed device, such as a computer, said current and/or voltage analysis circuit being capable of controlling voltage supply output parameters in relation to a trans-substrate voltage and current flow according to user-predefined procedures, such as turning off said voltage supply output once a user specified trans-substrate current threshold is exceeded, and/or said current and/or voltage analysis circuit being capable of controlling said means to apply energy.

56. The device according to any of claims 52 - 55, wherein said voltage supply is a regulated voltage supply that obtains feedback signals from the process of forming a structure, such as for example current flow and heat, and subsequently adjusts the voltage parameters, such as amplitude, frequency, and duty cycle in a predefined, preferably user-defined manner, so as to produce the desired structure.

57. The device according to any of claims 52 - 56, wherein said means to apply heat is an electric heating filament, preferably controlled by said control unit of claim 55.

58. The device according to any of claims 52 - 56, wherein said means to apply energy, preferably heat, is a laser or other focussed light source or high energy radiation source or a flame, for example from a micro torch.



59. The device according to any of claims 52 - 56, wherein said means to apply energy, preferably heat is an AC voltage supply connected to said at least two electrodes, or, if present to further sets of electrodes.

60. The device according to claim 59 wherein said AC voltage supply is combined with said voltage supply of claim 52, to one single voltage supply, capable of generating an AC voltage component which can cause an AC current sufficient to heat said substrate and, preferably, to cause a dielectric breakdown through said substrate.

61. The device according to any of claims 52 - 60, wherein a distance between the at least two electrodes is in the range from 0.01 to 60 mm, preferably 0.1 to 15 mm and more preferably between 0.5 to 8 mm.

62. The device according to any of claims 52 - 61, further comprising said electrically insulating substrate in a position substantially between said at least two electrodes and accessible to said means to apply heat.

63. The device according to any of claims 52 - 62, further comprising means to avoid electric arcs between said electrodes bypassing said substrate by ionizing the surrounding medium, e.g. air, such as rubber seals or glass plates tightly attaching to the substrate and effectively increasing the distance that an electric arc between the electrodes would have to take when bypassing said substrate.

64. The device according to any of claims 52 - 63, further comprising means for further modifying a surface of said substrate by a physical reaction initiated and/or maintained by the voltage and current used for forming said structure, or by a chemical reaction with an additional material that reacts with said surface of said substrate during the process of forming a structure.

65. The device according to claim 64, wherein said means for further modifying said surface of said substrate is a container for receiving said substrate and, additionally, a medium, such as a gas or liquid, surrounding said substrate.

66. The device according to any of claims 52 – 65, further comprising means to modify said structure formed, in a postprocessing step by heat application to said substrate such as to smoothen a substrate surface and/or to change the size of said structure.

67. The device according to any of claims 52 – 66, further comprising an electrically insulating substrate in which a structure is to be formed.

68. An electrically insulating substrate having a structure or an array of structures produced by the method according to any of claims 1-51

69. The substrate according to claim 68, wherein said structure is a hole having an aspect ratio greater than 1, preferably greater than 5, more preferably  $> 10$ , or wherein said structure is an array of such holes.

70. The substrate according to any of claims 68 – 69, wherein said substrate is made from a material selected from a group comprising carbon-based polymers, such as polypropylene, fluoropolymers, such as Teflon, silicon-based substrates, such as glass, quartz, silicon nitride, silicon based polymers such as Sylgard, semiconducting materials such as elemental silicon, wherein, preferably, said substrate is made from glass, quartz or silicon oxide or silicon nitride, or a mixture of any of the foregoing.

71. A device comprising a substrate according to any of claims 68 - 70 to support, capture or carry a biological object, such as a biological cell, or a lipid-based membranous object or structure.

72. The device, according to claim 71, wherein said substrate separates at least two fluid compartments which are accessed by electrodes in such a way that the fluid compartments are only connected through said hole of said substrate.

73. Use of the substrate according to any of claims 68 - 70 or the device according to any of claims 71 - 72 for patch clamp measurements, black lipid membrane measurements, in micro fluidic devices, or for performing nucleic acid hybridization experiments.